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Susceptibility to Activity-Based Anorexia

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College of William & Mary - Arts & Sciences

SUSCEPTIBILITY TO ACTIVITY-BASED ANOREXIA

A Thesis

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Master of Arts

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by

Eric Z. Stanley

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APPROVAL SHEET

This thesis is submitted in partial fulfillment of

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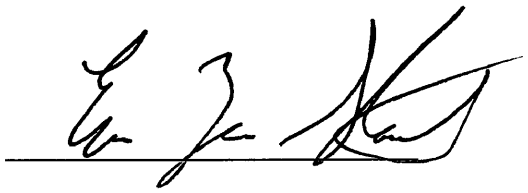
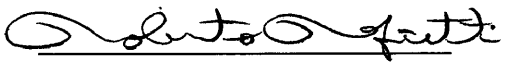
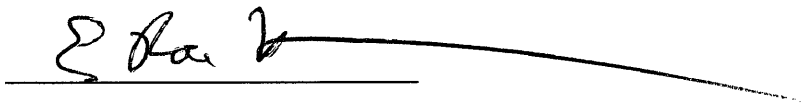


Eric Zane Stanley

Approved, July 1996



Roberto Refinetti



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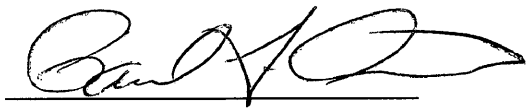

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The data were presented in a preliminary fashion to the Society for Neuroscience: Stanley, E.Z., Doerries, L.E., Rieg, T.S., & Aravich, P.F. (1992). Suprachiasmatic nucleus (SCN) lesions increase susceptibility to activity-based anorexia [Abstract]. Society for Neuroscience Abstracts, 64, 24.

This thesis is dedicated to my wife, Maria Antonia Stanley, whose love and constant support made completion of this thesis possible.

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ABSTRACT

A number of studies indicate that the suprachiasmatic nucleus (SCN) of the hypothalamus is the master oscillator of light-entrainable circadian rhythms. Its destruction results in the elimination of a variety of these rhythms. Studies also show that constant bright illumination disrupts circadian rhythms in numerous species. There is growing interest in the role of disrupted circadian rhythms in various disorders, including the eating disorder known as anorexia nervosa. Activity-based anorexia (ABA) is considered by many researchers to be a useful animal model for anorexia nervosa. It models the hyperactivity and weight loss that occur in the disorder, like many humans who are of normal or below normal weight and who simultaneously initiate a program of diet and strong exercise, rats which are also subjected to similar exercise and food restriction, increase activity levels, decrease food intake and lose weight. It is also an effective model for the effects of exercise stress in malnourished subjects in general. The purpose of this experiment was to determine if the two treatments known to disrupt light-entrainable circadian rhythms increase the rate of weight loss in male rats subjected to ABA. A 2 x 2 randomized factorial design was used. Factor A was lighting condition: a regular 12/12 hour light/dark (LD) cycle or a 24 hr constant bright illumination (LL) condition. Factor B was surgery: sham SCN lesions or SCN lesions. The independent variables were imposed and then all rats were subjected to the ABA syndrome.

Results indicated that the SCN-lesioned LD and SCN-lesioned LL groups lost weight more rapidly than normal controls subjected to the syndrome as assessed by days to reach a 25 percent weight-loss criterion; the sham SCN-lesioned LL group was intermediate in rate of weight loss. These differences resulted in a 52-56 percent increase in rate of weight loss for the circadian-manipulated groups compared to controls. The increased rate of weight loss in the experimental groups was not related to decreased food intake, increased running or changes in relative adrenal gland weight. In fact, wheel running was actually decreased in experimental groups when the data were averaged across syndrome days, which replicates the well-established suppression of running produced by SCN lesions.

Since circadian rhythms have a number of effects on energy metabolism, it was proposed that the adverse weight-loss effects of the circadian manipulations were due to a decrease in metabolic efficiency during the syndrome. It was argued that this effect was independent of the glucocorticoids. The intermediate effect on weight loss in the sham SCN-lesioned LL group was attributed to a less effective disruption of the relevant circadian rhythms. Finally, it was proposed that the effects of disrupted light-entrainable circadian rhythms should now be investigated in malnourished people subjected to exercise stress and in patients suffering from anorexia nervosa in particular.

The Effects of Bilateral Suprachiasmatic Nucleus Lesions and Constant Illumination on Susceptibility to Activity-Based Anorexia

INTRODUCTION

Anorexia nervosa is a significant disorder resulting in severe weight loss and potentially death. The mortality rate of diagnosed anorexics is estimated between 5 and 21 percent (Mrosovsky & Sherry, 1980; Silverman, 1983; Stricker & Andersen, 1980; Yates, 1989). Death most often results from starvation, suicide or electrolyte imbalance (APA, 1994). The disorder can range in occurrence from 1 in 150 in Sweden to 1 in 90 for adolescent females in England (Szmulker, 1983). The rate of incidence for anorexia is significantly higher in females than in males (Crisp, 1983; Garfinkel & Garner, 1983). A variety of factors are perturbed in anorexia nervosa (Yates, 1989), including circadian rhythms (Brambilla, Ferrari, Petraglia, Fachchine, Catalano, & Genazzani, 1991; Ferrari, Franschini, & Brambilla, 1990; Katz, Boyar, Roffwarg, Hellman, & Weiner, 1978).

According to the DSM-IV (APA, 1994) an important diagnostic criterion for anorexia nervosa in humans is a 15 percent body weight-loss. Along with the weight loss caused by decreased food intake, anorexics show symptoms of hyperactivity (Yates, Leehey, & Shisslak, 1983). In fact, excessive physical exercise and reduced food-intake are significant risk factors for anorexia nervosa (APA, 1994; Yates, 1989). The DSM-IV defines two subtypes of anorexia nervosa: the restricting type describes presentations in which weight loss is accomplished primarily through dieting, fasting and exercise; and the binge-eating/purging type where the individual is regularly engaged in binge eating or

purging or both (APA, 1994). Types of purging behaviors include self-induced vomiting, laxative abuse and exercise.

According to Epling and Pierce (1991) chaotic dieting, excessive activity and physiological abnormalities are associated with the human condition of anorexia nervosa. Their contention is that a fundamental feature of anorexia nervosa is activity anorexia. Activity anorexia is defined and occurs when there is a decline in food intake that increases physical activity. Furthermore, as physical activity becomes excessive, the food consumption is reduced further, and this reduction in the caloric intake leads to more activity. This vicious feedback cycle can lead eventually to death.

Current research suggests a useful animal paradigm to examine the interaction between excessive exercise and restricted feeding (Beumont, 1984; Yates, 1989). According to this paradigm post adolescent rats are simultaneously introduced to a restricted feeding schedule and given the opportunity to voluntarily exercise. The effect of such a manipulation is that they progressively increase their activity, eat less and lose body weight compared to rats simply placed on restricted feeding schedules (Routtenberg & Kuznesof, 1967; Spear & Hill, 1962). This effect has been replicated by numerous investigators (Doerries, Stanley, & Aravich, 1991; Epling, Pierce, & Stephan, 1983; Kanarek & Collier, 1983) and has been named activity-based anorexia (ABA) (Epling et al., 1983).

Pierce and Epling (1994) further suggest, in order to extend this activity-based anorexia model to anorexia nervosa, that there are five levels of functional

similarity: 1. Excessive physical activity is associated with anorexia nervosa in humans. Kron, Katz, Gorzynski, & Weiner (1978) conducted an extensive retrospective study that examined hospitalized anorexics and concluded that hyperactivity is a central feature of anorexia nervosa. Various other investigators support this view (Yates, Leehay, & Shisslak, 1983; Yates, 1989). 2. Increasing physical activity reduces food consumption (Edholm, Flecher, Widdowson, & McCance, 1955; Johnson, Mastropaolo, & Wharton, 1972). This effect occurs when activity increases against an individuals base activity rate and subsides when their activity stabilizes (Epling & Pierce 1984). 3. Lower food consumption increases physical activity in both people (Russell-Davis, 1951) and rats (Boer, Epling, Pierce, & Russell, 1990). 4. The anorexia in the animal model develops in a similar way to the anorexia in patients. For example, in the laboratory, food restriction generates excessive activity that in turn interferes with eating. Similar patterns occur in anorexic patients (Beumont, 1991; Katz, 1986). 5. Reproductive function is disrupted for physically active rats, anorexic patients and athletes. In particular, females with anorexia nervosa who are hyperactive, have menstrual cycle problems (Kaye, Picker, Naber, & Ebert, 1982) as is the case with the estrous cycle of food-deprived female rats that run excessively on an activity wheel (Watanabe, Hara, & Ogawa, 1992). In addition to these variables, another similarity between anorexia nervosa and the animal model is an elevation in the adrenal glucocorticoids (Yates, 1989; see Watanabe, Hara, & Ogwa, 1992).

Researchers at the Hampton Veterans Affairs Medical Center (Hampton, VA) and Christopher Newport University (Newport News, VA) have been systematically investigating the animal syndrome (see Aravich, in press, for a review). For example, they determined ABA's relationship to the endogenous opiods, vasopressin, glucoprivic feeding, immune function, gastric stress ulcers and gender factors (Aravich, Doerries, Farrar, Downing, Elhady, Metcalf, & Johnson, 1990; Aravich, in press; Aravich, Stanley, & Doerries, 1995; Doerries, Stanley, & Aravich, 1991).

They have also collected data implicating light-entrainable circadian rhythms to ABA. For example, they determined that constant bright illumination affects susceptibility to ABA (Stanley, Doerries, Rieg, & Aravich, 1991). This is a well established technique to abolish circadian rhythms in rats (Glantz, 1967; Richter, 1971; Zucker, 1974). Male and female rats were compared. An adverse effect of constant bright illumination was shown in female but not male rats. The lack of significance in the males may have been the result of a floor effect caused by their lower weight at the start of the experiment. The adverse effects of constant illumination were not explained by an increase in wheel activity or a decrease in food intake since there were no statistical differences between lighting conditions within each gender. Instead, they were attributed to changes in metabolic efficiency. Further research is needed to determine the effects of circadian rhythm perturbations on ABA. This includes manipulations of the suprachiasmatic hypothalamic nucleus.

The suprachiasmatic nucleus (SCN) of the hypothalamus is the master oscillator for light-entrainable circadian rhythms since its destruction results in the elimination of a variety of rhythms (Rusak, 1989; Rusak & Bina, 1990; Rusak & Zucker, 1977). Specific rhythms affected include locomotor activity (Stephan & Nunez, 1977; Stephan & Zucker, 1972), drinking (Stephan & Nunez, 1977; Stephan & Zucker, 1972; Van Den Pol & Powley, 1979), feeding (Nagai, Nishio, Nakagawa, Nakamura, & Fukuda, 1978), sleep wakefulness (Stephan & Nunez, 1977; Ibuka & Kawamura, 1975; Ibuka, Inouye, & Kawamura, 1977) and temperature (Stephan & Nunez, 1977; Krieger, Hauser, & Krey, 1977). The effects of SCN lesions on susceptibility to ABA have not been examined.

The purpose of this study was to determine if SCN lesions adversely affect susceptibility to ABA. For the purposes of comparison, constant bright illumination was also used. Susceptibility to ABA was defined as the number of days required to lose 25 percent of original body weight. We have used this measure of susceptibility in numerous other published studies (see Aravich, in press). To determine if any observed changes were correlated with the glucocorticoids, relative adrenal weight, which is a crude index of glucocorticoid secretion (see Aravich, in press), was also evaluated. It was predicted that both circadian rhythm manipulations will increase susceptibility to ABA. Confirmation of this prediction will have important clinical implications and will raise the possibility that disrupted circadian rhythms may increase the adverse

effects of exercise stress in malnourished subjects in general and in patients suffering from anorexia nervosa in particular.

METHOD

Subjects

The subjects were 48 male Harlan Sprague Dawley rats. Male rats were used to control for cyclic changes in reproductive hormone status. There were 10-14 rats per treatment condition. The animals were approximately 50 days old upon arrival to the animal colony. The rats were habituated to the new environment and lighting schedules, subjected to surgery and given a post-operative recovery period. They weighed approximately 293-303 grams and were 65-67 days old at the start of the ABA phase of the experiment.

Apparatus

All 48 rats were housed in Wahmann type running wheels with connecting side cages (see Appendix A). The wheels and side cages, as one apparatus, are manufactured by Lafayette Instruments Co.; Lafayette, IN; (model no. 86041). The diameter of the wheel is 35.6 cm and the width is 10.8 cm. The side cage is 25.4 cm x 15.2 cm x 12.7 cm. Passage between the running wheel and the side cages was controlled by a sliding door.

Neurosurgery was performed on a Kopf stereotaxic instrument (Kopf Instruments; Tujunga, CA; model no. 1504). Electrolytic lesions were made with a Stoelting Lesion Producing Device by C. H. Stoelting Co.; Chicago, IL; (model no. 58040). Histological confirmation of the lesion site involved frozen nissl-

stained sections (30 microns thick) cut on a cryostat (American Optical; Buffalo, NY; model no. 520) and viewed through a microscope (Carl Zeiss Inc.; Thornwood, NY; model no. MC638). The nissl-stain used cresyl violet acetate (Eastman Kodak; Rochester, NY).

Procedure

The rats were assigned to four treatment conditions according to a 2 x 2 randomized factorial design. The four treatment conditions were: sham-lesioned/normal light-dark schedule (LD), SCN-lesioned/normal light-dark schedule (LD), sham-lesioned/constant bright illumination (LL), and SCN-lesioned/constant bright illumination (LL).

All animals were individually housed in the side cages of the running wheels and were allowed to habituate to the new environment and the respective lighting schedules for 7-10 days. Animals in the normal lighting conditions were on a 12/12 hr LD cycle (light phase: 0700-1900 hours) and were maintained on this schedule throughout the experiment. Animals in the LL conditions were maintained on constant illumination throughout the experiment. Lighting was provided by two overhead fluorescent 4-foot fixtures and was estimated to be 200 Lux. All rats had free access to food and water throughout the habituation and post operative recovery periods (see below), but access to the running wheel was blocked. They were fed Purina Rat Chow, number #5001, and had water available from standard water bottles.

After habituation to the lighting schedules, the animals were anesthetized with an i.p. injection of ketamine-xylazine (60.0 mg/kg body weight of ketamine, Ketaset, Aveco Co.; Fort Dodge, IA; 7.5 mg/kg body weight of xylazine, Rompun, Mobay Corporation; Shawnee, KS) and mounted in the stereotaxic instrument. Bilateral electrolytic SCN lesions were made by passing an anodal DC current (1 mA for 15 seconds) through a stainless steel electrode (no. 00 insect pin) insulated with four coats of epoxy except for 0.5 mm of the tip. The cathode was attached to the pinna with a small alligator clamp. The SCN was located using the following coordinates: 1.3 mm posterior to bregma, +0.3 mm lateral to the midline and 9.1 mm ventral to the top of the skull (according to the atlas of Paxinos and Watson, 1986). The skull was level between bregma and lambda. Bilateral sham lesions were performed by positioning the electrode using the same parasagittal and coronal coordinates. However it was lowered to a depth one millimeter higher (final position: -8.1 mm beneath the skull surface) than the actual lesion depth. No current was passed through the electrode in the sham-lesioned controls. A 7-10 day recovery period was allowed following surgery for each of the four groups. During the last three days of this period, the circadian distribution of food intake was determined to confirm the efficacy of the SCN lesions and constant illumination.

The ABA portion of the experiment began the next day at 1330 hours. This is defined as syndrome day zero. This time corresponds to the end of the 1.5 hr daily restricted-feeding periods that began the next day. The restricted-

feeding periods occurred during the middle of the LD cycle, i.e., 1200-1330. The animals were weighed to determine original body weight at syndrome onset. Food was then removed from the cages and the rats were given access to the running wheels for the next 22.5 hours. At 1200 hours of the following day, the animals were weighed to determine their percent of original body weight loss and given a measured amount of food on the floor of the side cages. The number of wheel revolutions was recorded to determine the total activity for that day. Access to the running wheel was blocked during feeding. After 1.5 hours of food access the food was removed along with spillage and weighed to assess the amount of food consumed during the feeding period. These data were recorded as Day 1. The door to the wheel was then opened to allow free access to the wheel for the next 22.5 hours. This procedure was followed until the rat lost 25 percent of its original body weight.

Once the animals reached the weight loss criterion they were sacrificed by decapitation to permit potential analysis of selected areas of the brain, pituitary and plasma. After sacrifice an adrenal gland was removed and weighed. All adrenal gland weights were expressed relative to total body weight. The region of the brain containing the SCN was blocked and immersion fixed in a 10% buffered formalin solution for histological verification of the lesion site.

Design and Statistical Analysis

The data were analyzed by a 2 x 2 randomized factorial analysis of variance. Factor A was lighting condition (12/12 hour LD cycle or LL) and

factor B was surgery (sham SCN-lesioned or SCN-lesioned). Post hoc comparisons were performed by Tukey t-tests of all possible comparisons (GB Stat statistics program, Dynamic Microsystems, Inc.; Silver Springs, MD).

The final data analysis included animals with histologically verified bilateral SCN lesions. Of the 48 animals that under went surgery, 44 survived. One died at the time of the injection of the ketamine-xylazine anesthesia, one was found dead early the next morning after surgery, and the other two animals were unable to gain weight and, after several days of substantial weight loss, were sacrificed. Histological examination of the lesion sites revealed total ablation of the SCN in 17 of the 24 animals in the lesion groups. Four other animals had either some lateral or bilateral sparing of the anterior portion of the SCN but were kept in the data analysis. Three of the 24 animals were excluded from the analysis because the SCN was spared either unilaterally or bilaterally. . Tracings of typical complete and spared SCN lesion are included in Appendix B After exclusion, the four group sizes were as follows: 10 rats in the sham SCN-lesioned LD condition, 12 rats in the SCN-lesioned LD condition, 10 rats in the sham-lesioned LL condition, and 9 rats in the SCN-lesioned LL condition.

RESULTS

Figure 1 shows the mean weights for the animals in each of the four conditions on day zero of the syndrome. The means ranged from 293.0 SE $\pm$ 3.9 to 303.6 SE $\pm$ 2.1 grams. No significant lighting condition effect, surgery effect or lighting condition x surgery interaction effect was observed. Pairwise comparisons also failed to reveal any differences.

Insert Figure 1 about here

To determine if light-entrainable circadian rhythms were disrupted prior to the weight-loss syndrome, the percentage of total food intake consumed at night after surgery was determined (see Figure 2). A 2-way ANOVA revealed a significant lighting condition effect, $F\ (1,40) = 222.62$, $p < .0001$, and a surgery effect, $F\ (1,40) = 292.90$, $p < .0001$. A lighting condition x surgery interaction effect was also observed, $F\ (1,40) = 198.11$, $p < .0001$. Tukey t-tests indicated that the sham SCN-lesioned LD rats differed from all other groups in the percentage of food consumed at night after surgery.

Insert Figure 2 about here

In order to determine whether food intake differed for the four groups during the syndrome, food intake was analyzed using two different methods. The

first method examined the amount of food consumed during the 1.5hr feeding period prior to sacrifice (see Figure 3), when the syndrome was at its maximum. The second method examined the average amount of food consumed throughout the entire weight-loss syndrome (see Figure 4). The data were then analyzed by separate 2-way ANOVA's. No significant lighting condition effect, surgery effect or lighting condition x surgery interaction effect was observed for either measure. Pairwise comparisons (Tukey t-tests) of the data also failed to reveal any differences for these measures.

Insert Figures 3 & 4 about here

To determine whether wheel activity differed for the four groups during the syndrome, wheel revolutions were analyzed using two different methods. The first examined the mean wheel revolutions, during the 24hr period prior to sacrifice, when the syndrome was at its maximum (see Figure 5); the second method examined the average wheel revolutions by each animal across the entire experimental period (see Figure 6). The data were then analyzed by separate 2-way ANOVA's. No significant lighting condition effect, surgery effect or lighting condition x surgery interaction effect was observed for mean wheel revolutions during the 1.5 hr feeding period prior to sacrifice. By contrast, analysis of the average number wheel revolutions across the entire experimental period revealed a significant surgery effect, $F(1,40) = 18.67$, $p < .0003$, but no

lighting condition effect or lighting condition x surgery interaction effect. Pairwise comparisons of the data further revealed that the rats in all groups with circadian disruptions ran less than the sham SCN-lesioned LD control.

Insert Figures 5 & 6 about here

To determine if the circadian distribution of activity was disrupted during the syndrome the percentage of total wheel revolutions at night during the 24hr period prior to sacrifice was analyzed (see Figure 7). A 2-way ANOVA revealed a significant lighting condition effect, F (1,40) = 26.78, $p < .0001$, and a surgery effect, F (1,40) = 35.17, $p < .0001$. A lighting condition x surgery interaction effect was also observed, F (1,40) = 41.49, $p < .0001$. Pairwise comparisons (Tukey t-tests) of the data further revealed that the rats in all groups with circadian disruptions had less of a percentage of total activity at night than the sham SCN-lesioned LD control.

Insert Figures 7 about here

To determine if the observed effects were related to adrenal gland changes, relative adrenal weights were assessed for all groups (see Figure 8). A 2-way ANOVA revealed no significant lighting condition effect, surgery effect or

lighting condition x surgery interaction effect. Pairwise comparisons (Tukey t-tests) of the data also failed to reveal any differences.

Insert Figure 8 about here

Finally, to determine if the circadian manipulations altered susceptibility to ABA, the mean number of days required to reach the 25 percent weight-loss criterion in each of the four conditions was analyzed (see Figure 9). A 2-way ANOVA revealed a significant lighting condition effect, $F(1,40) = 17.76$, $p < .0003$, and surgery effect, $F(1,40) = 85.57$, $p < .0001$. A lighting condition x surgery interaction effect was also observed, $F(1,40) = 29.31$, $p < .0001$. Tukey t-tests indicated that the SCN-lesioned LD, and the SCN-lesioned LL rats were most susceptible to ABA and that the sham SCN-lesioned LL rats were intermediate in susceptibility compared to the sham SCN-lesioned LD rats. For example, rats in the SCN-lesioned LD condition required an average of 4.4 fewer days to lose 25 percent of their original body weight compared to the control condition (i.e., the sham SCN-lesioned LD group).

Insert Figure 9 about here

DISCUSSION

The purpose of the present investigation was to determine if two treatments known to disrupt circadian rhythms affect susceptibility to ABA in male rats. It was predicted that SCN lesions and constant bright light would increase susceptibility to ABA. The results supported the hypothesis: Rats subjected to each treatment condition were more susceptible to activity-based anorexia. In fact they were 52-56 percent more vulnerable to the syndrome than untreated controls.

This experiment also shows that the increased rate of weight loss was not due to decreased food intake or increased wheel activity. To the contrary, the groups with the circadian rhythm treatments actually ran less across the entire experiment than normal controls. This reduction in wheel running is consistent with the well-known suppression of running that occurs with SCN lesions in other contexts (Refinetti, 1995; Stephan & Zucker, 1972). Independent verification that the treatments were effectively implemented was provided by the histological verification of the lesions, by the disruption of the circadian distribution of food intake prior to the syndrome, and by the disruption of the circadian distribution of wheel running during the syndrome. The next step would be to identify the specific rhythm or rhythms that affect ABA.

Since the increased rate of weight loss in the ABA syndrome cannot be easily explained by an increase in wheel activity or a decrease in food intake, another explanation in needed. One possibility is that the disruption of certain

rhythms by either lesioning the SCN or by constant bright illumination produces a decrease in metabolic efficiency. This could be due to an increase in the metabolic cost of running and/or a decrease in the efficiency of caloric utilization. Numerous systems that modulate these functions, including the sympathetic nervous system, brown adipose tissue and pancreatic hormones, have been linked to the SCN (Nagai & Nakagawa, 1992).

This investigation also demonstrates that animals with constant bright illumination alone are less adversely affected than animals with SCN lesions in terms of susceptibility to ABA. One possible explanation for this difference is that the constant bright illumination may have less effectively disrupted the circadian rhythms relevant to this syndrome even though it had a comparable effect on the circadian distributions of wheel running and food intake. Further research is needed with different levels of light intensity to determine the specific dose-response relationship to maximally affect susceptibility to the syndrome.

Another finding of this investigation is that the adverse effect of disrupted circadian rhythms was not correlated with changes in relative adrenal weight. This is consistent with the possibility that excess glucocorticoid secretion does not mediate the effect. A direct measurement of glucocorticoid level and sensitivity is needed to confirm this conclusion.

Yet another finding of this experiment is that the brain intact ABA rats housed under a normal light/dark cycle maintained a nocturnal distribution of wheel running which indicates that the syndrome cannot override the nocturnal

running rhythm of rats. This replicates previous findings (Beneke, Schulte, & Van der Tuig, 1995). Whether or not the syndrome is able to override other circadian rhythms is an empirical question. Its impact on food-entrainable rhythms, which are independent from light entrainable rhythms (Armstrong, 1980; Mistlberger, 1990), also remains to be determined.

One final result of this experiment is that a failure to see an adverse effect of constant illumination on the male rats in an earlier LL study (Stanley, Doerries, Rieg, & Aravich, 1991) was indeed due to a floor effect. The correction of this floor effect was achieved by using heavier animals at the onset of the syndrome. Thus, the investigation reveals the utility of using animals with a heavier starting weight for investigating variables that impact upon the weight-loss syndrome.

While susceptibility to ABA was increased in the groups that had their light-entrainable circadian rhythms disrupted, it is not clear which rhythms were responsible for the significant difference. While most studies agree that the SCN is the master oscillator of light-entrainable circadian rhythms, they do not eliminate the possibility that the SCN is only one component in a more complex multioscillatory system that acts as the circadian pacemaker. In fact, data from other studies support the presence of a multioscillatory system that acts in this capacity. Three examples include, (1) the phenomenon of splitting, which occurs when many species are housed in conditions of constant bright illumination (Pickard & Turek, 1982); (2) the spontaneous internal desynchronization of

certain rhythms in humans (Zulley & Campbell, 1985); and (3) observations of a food-entrainable oscillator that is evident in rats with complete SCN lesions (see above). One possible future study might be to examine the role of the pineal gland, which produces and regulates the hormone melatonin. Melatonin levels are known to be perturbed in humans with anorexia nervosa (Ferrari, Franschini, & Brambilla, 1990) and melatonin injections can entrain certain circadian rhythms in some rat strains. Melatonin has also been implicated in various aspects of metabolism and energy expenditure (Saarela & Reiter, 1994). Perhaps the study of susceptibility to ABA in rats that have their pineal gland removed or are subjected to direct injections of melatonin might help us in identifying the specific rhythms that increase the specific rhythms that play a role in the increased vulnerability to the syndrome.

In conclusion, there is growing interest in the relationship of circadian rhythms to physical health (Van Cauter & Turek, 1990), metabolism (Nagai & Nakagawa, 1992) and anorexia nervosa (Study Group on Anorexia Nervosa, 1995). This investigation demonstrates that two treatments that disrupt light-entrainable rhythms markedly increase vulnerability to the weight-loss syndrome produced by exercise-stress in malnourished rats. Since the effect was not due to increased running or decreased food intake it was attributed to a decrease in metabolic efficiency. The possibility that disrupted light-entrainable circadian rhythms worsen the weight-loss effects of exercise-stress in malnourished subjects

in general, and in patients suffering from anorexia nervosa in particular, should now be investigated.

Figure Caption

Figure 1. Mean ± SE body weight (g) at the start of the weight-loss syndrome for the 12/12hr LD and 24hr LL conditions. The open bars represent the sham SCN-lesioned groups and the closed bars represent the SCN-lesioned groups.

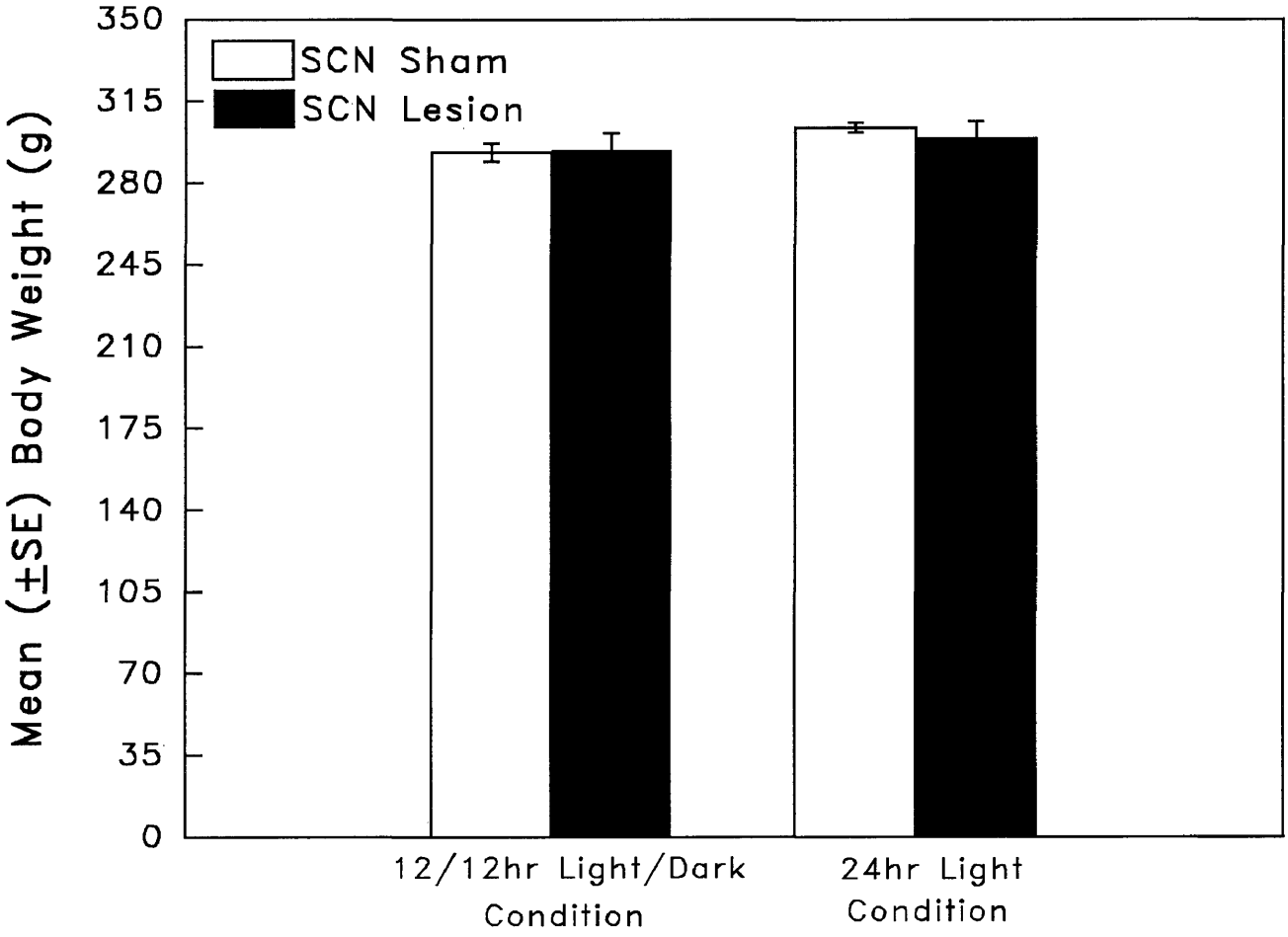

SCN Sham
SCN Lesion
Mean (±SE) Body Weight (g)
350
315
280
245
210
175
140
105
70
35
0
12/12hr Light/Dark Condition
24hr Light Condition

Figure Caption

Figure 2. Mean $\pm$ SE percentage of total food intake consumed at night after surgery for the treatment conditions indicated in Figure 1. (* $p < .05$, differs significantly from all other groups)

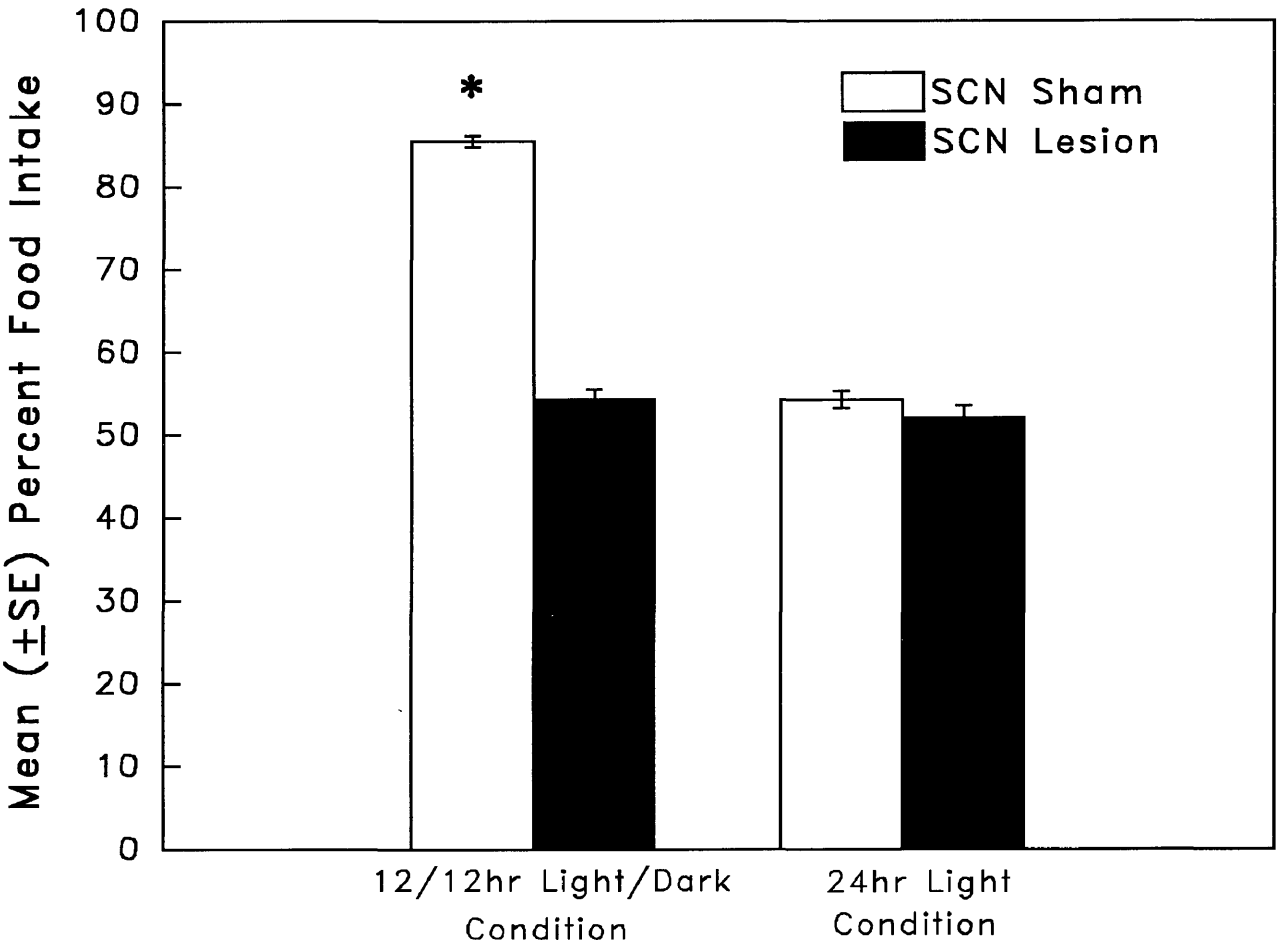

100
90
80
70
60
50
40
30
20
10
0
Mean (±SE) Percent Food Intake
*
SCN Sham
SCN Lesion
12/12hr Light/Dark
Condition
24hr Light
Condition

Figure Caption

Figure 3. Mean ± SE food intake (g) during the 1.5hr feeding period prior to sacrifice for the treatment conditions indicated in Figure 1.

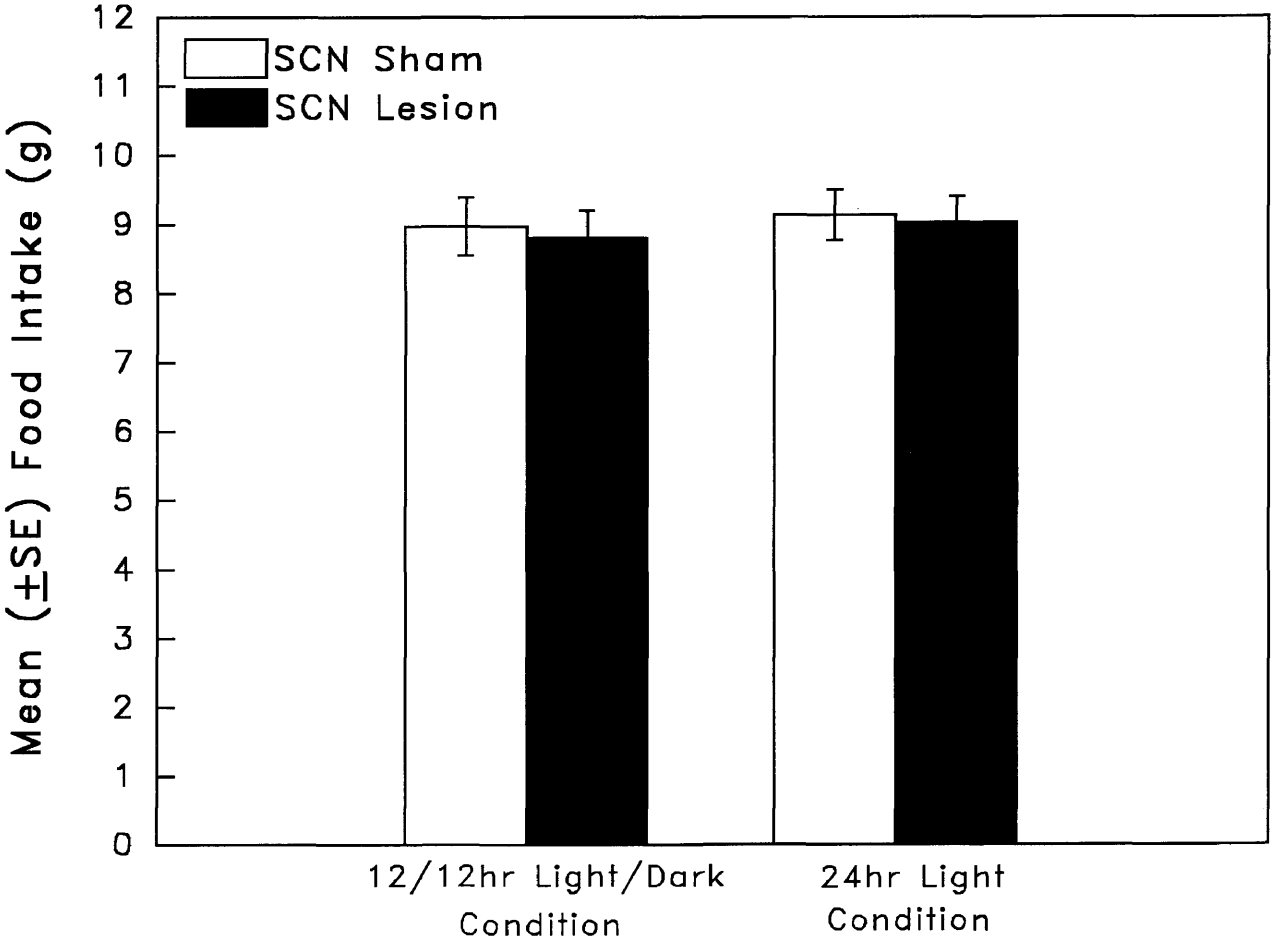

SCN Sham
SCN Lesion
Mean (±SE) Food Intake (g)
0
1
2
3
4
5
6
7
8
9
10
11
12
12/12hr Light/Dark Condition
24hr Light Condition

Figure Caption

Figure 4. Mean ± SE food intake (g) for the 1.5hr feeding period averaged across the entire syndrome for the treatment conditions indicated in Figure 1.

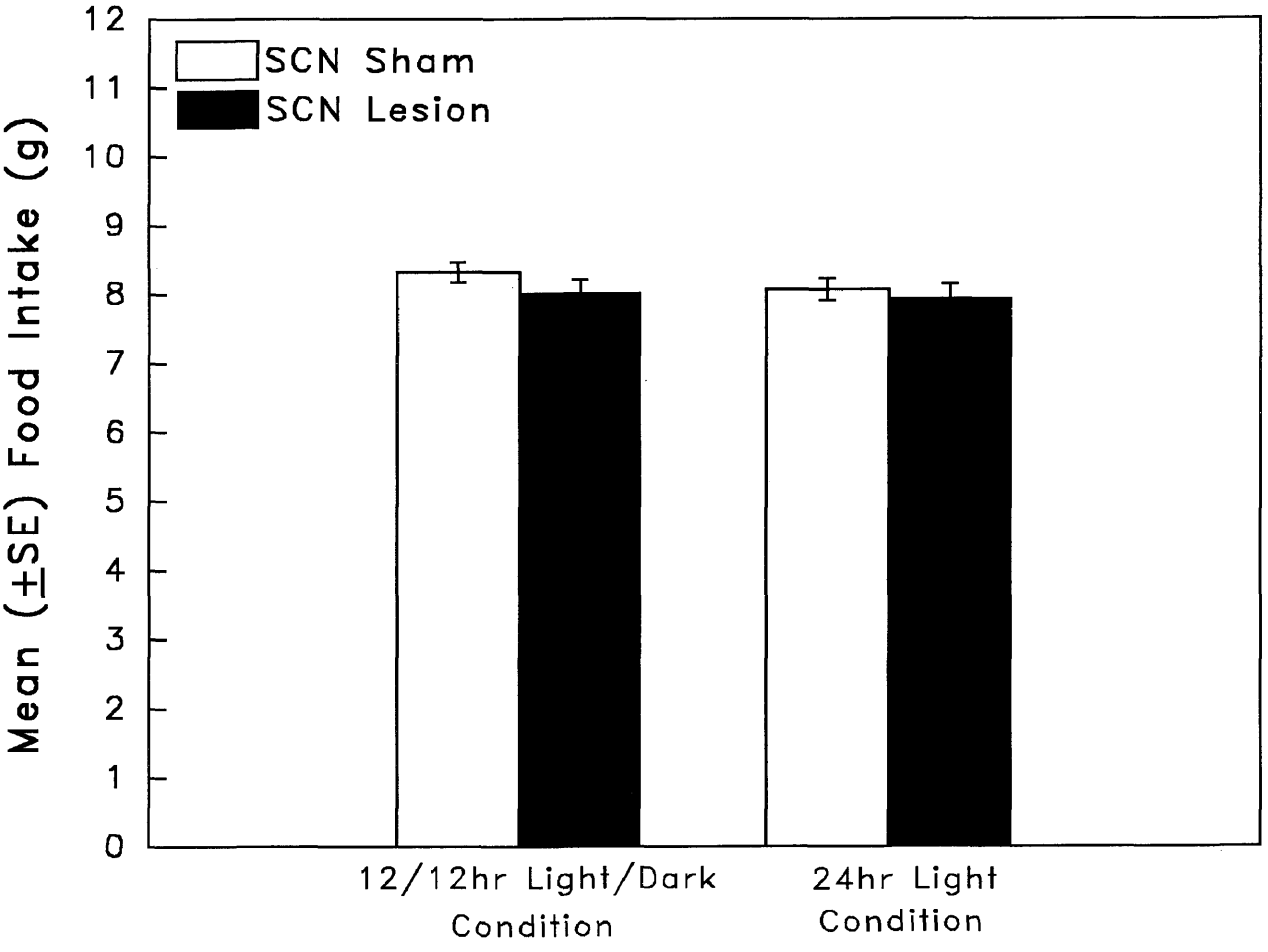

SCN Sham
SCN Lesion
Mean (±SE) Food Intake (g)
0
1
2
3
4
5
6
7
8
9
10
11
12
12/12hr Light/Dark Condition
24hr Light Condition

Figure Caption

Figure 5. Mean ± SE number of wheel revolutions during the 24hr period prior to sacrifice for the treatment conditions indicated in Figure 1.

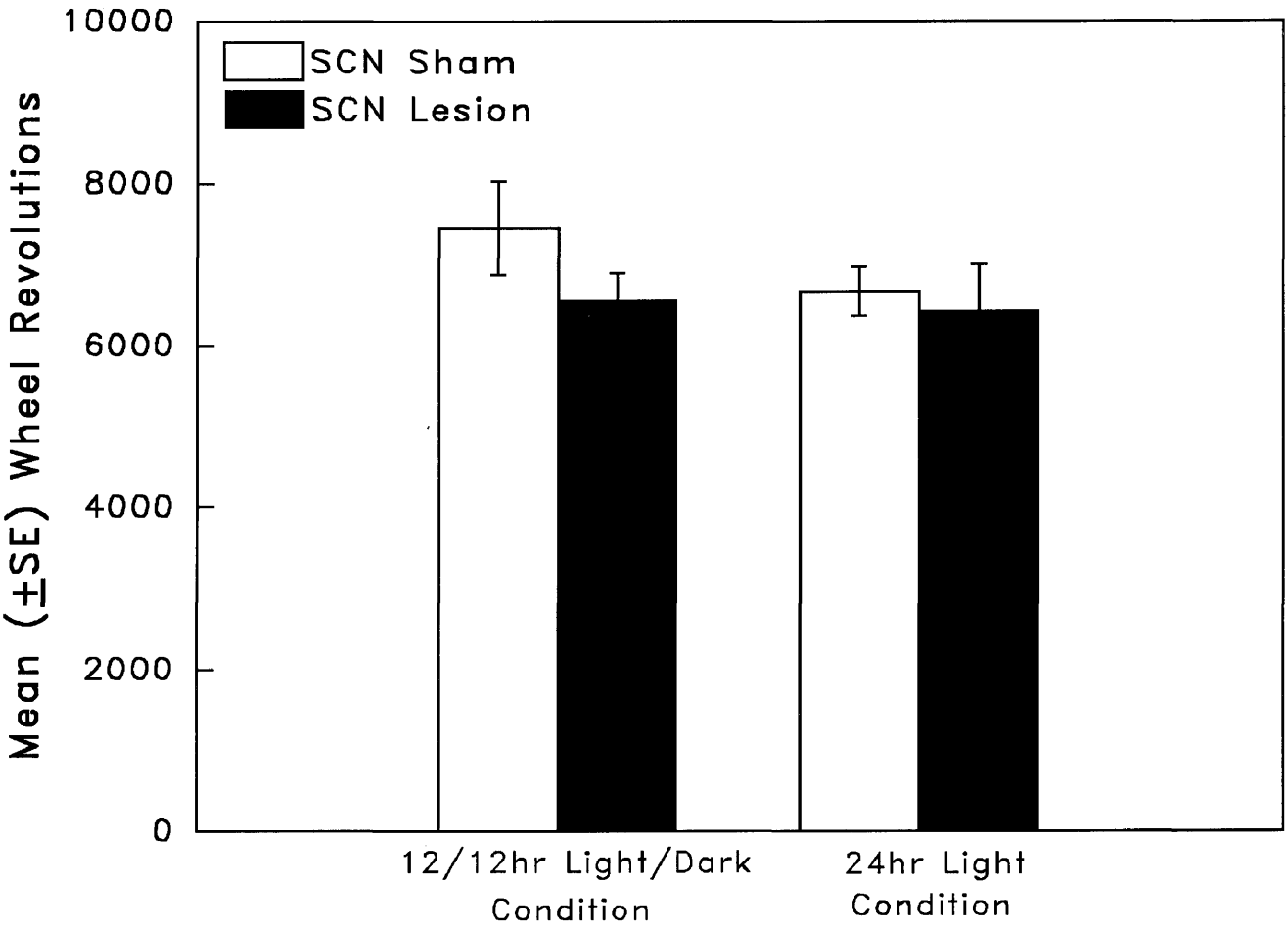

10000
8000
6000
4000
2000
0
Mean (±SE) Wheel Revolutions
SCN Sham
SCN Lesion
12/12hr Light/Dark
Condition
24hr Light
Condition

Figure Caption

Figure 6. Mean $\pm$ SE number of daily wheel revolutions across the entire syndrome for the treatment conditions indicated in Figure 1. (* $p < .05$, differs significantly from all other groups)

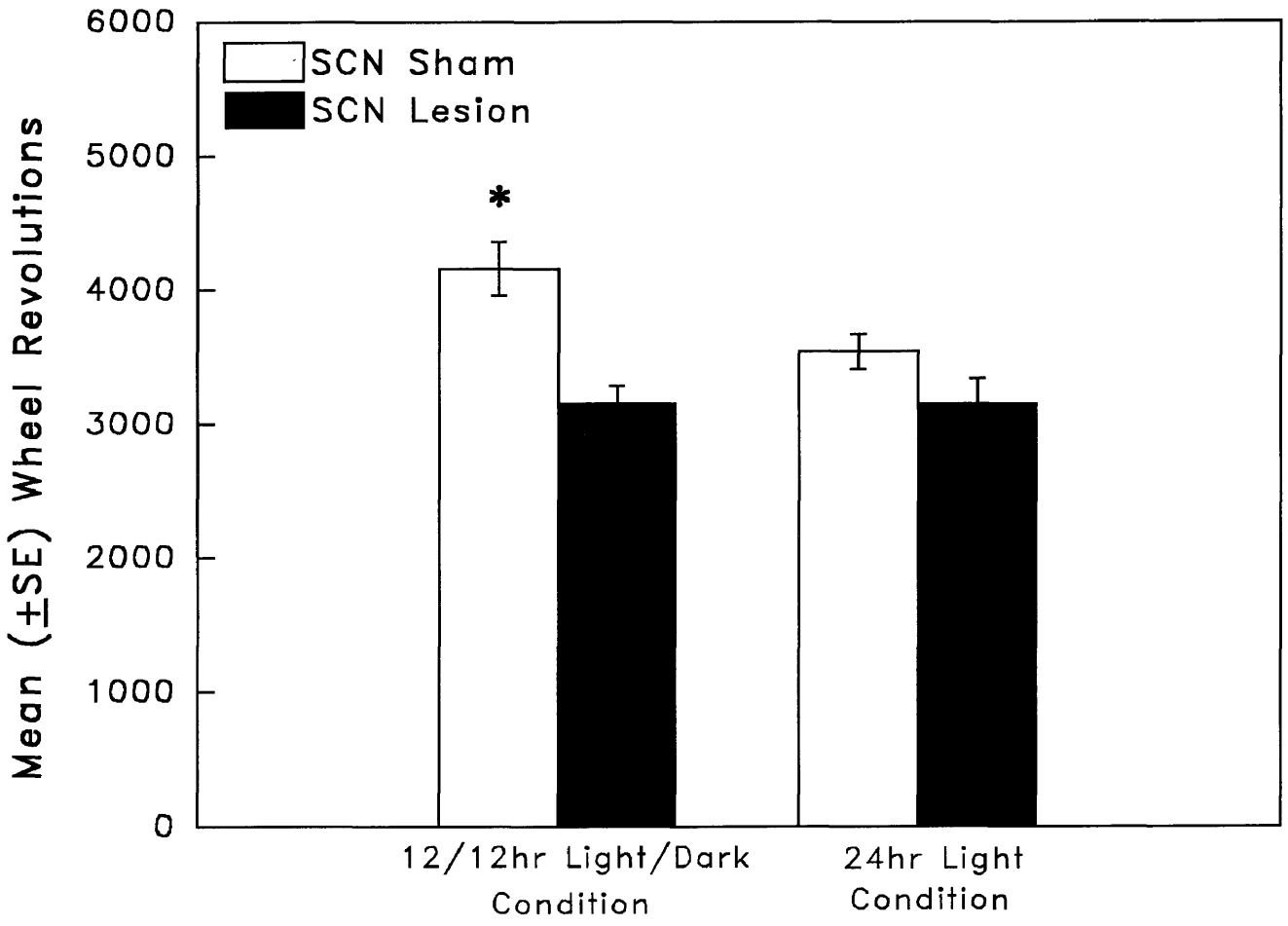

6000
5000
4000
3000
2000
1000
0
Mean (±SE) Wheel Revolutions
SCN Sham
SCN Lesion
*
12/12hr Light/Dark
Condition
24hr Light
Condition

Figure Caption

Figure 7. Mean $\pm$ SE percentage of total wheel revolutions at night during the 24hr period prior to sacrifice for the treatment conditions indicated in Figure 1. (* $p < .05$, differs significantly from all other groups)

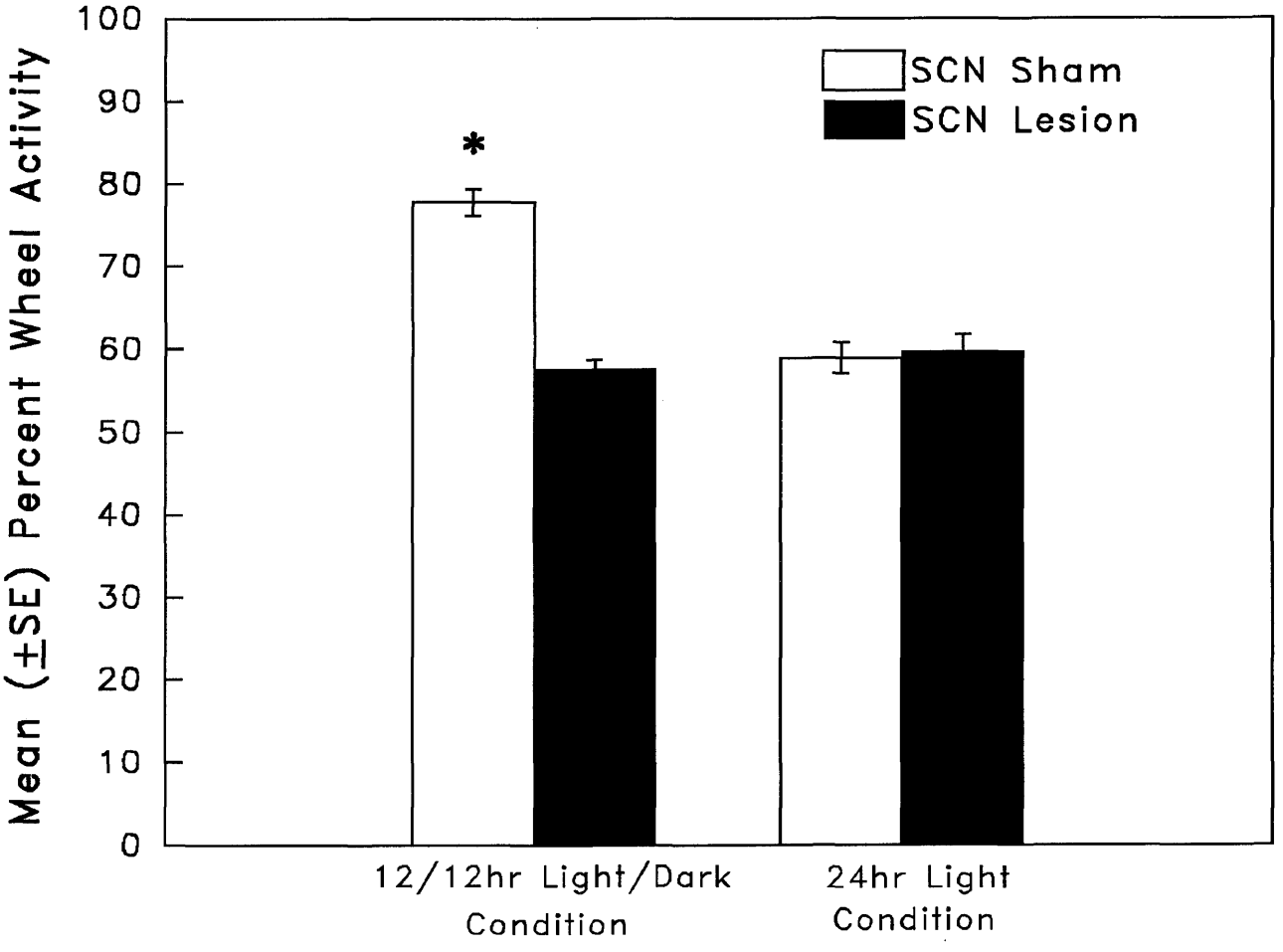

Mean (±SE) Percent Wheel Activity
100
90
80
70
60
50
40
30
20
10
0
SCN Sham
SCN Lesion
*
12/12hr Light/Dark Condition
24hr Light Condition

Figure Caption

Figure 8. Mean ± SE unilateral adrenal weight (g/kg body weight) at sacrifice for the treatment conditions indicated in Figure 1.

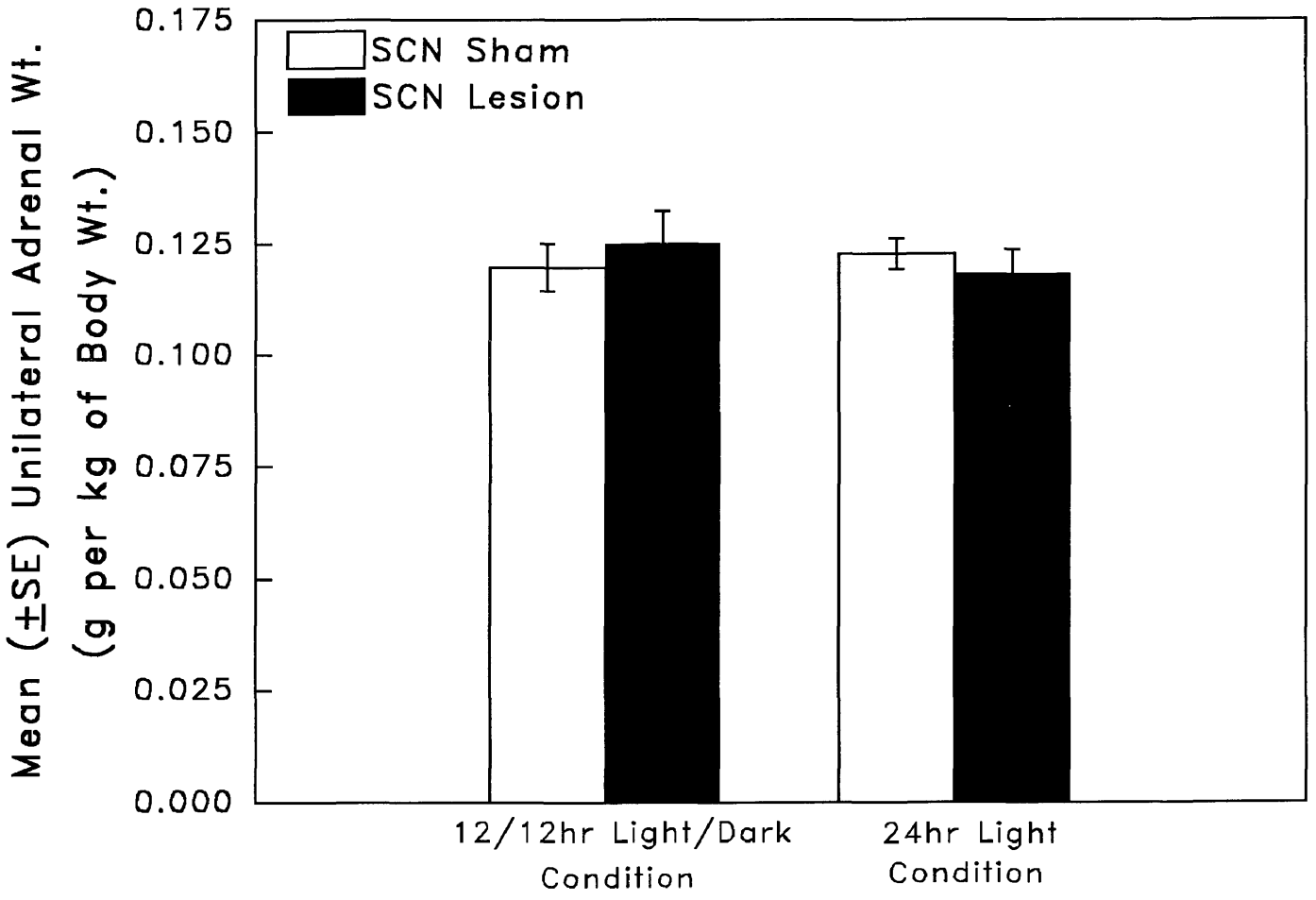
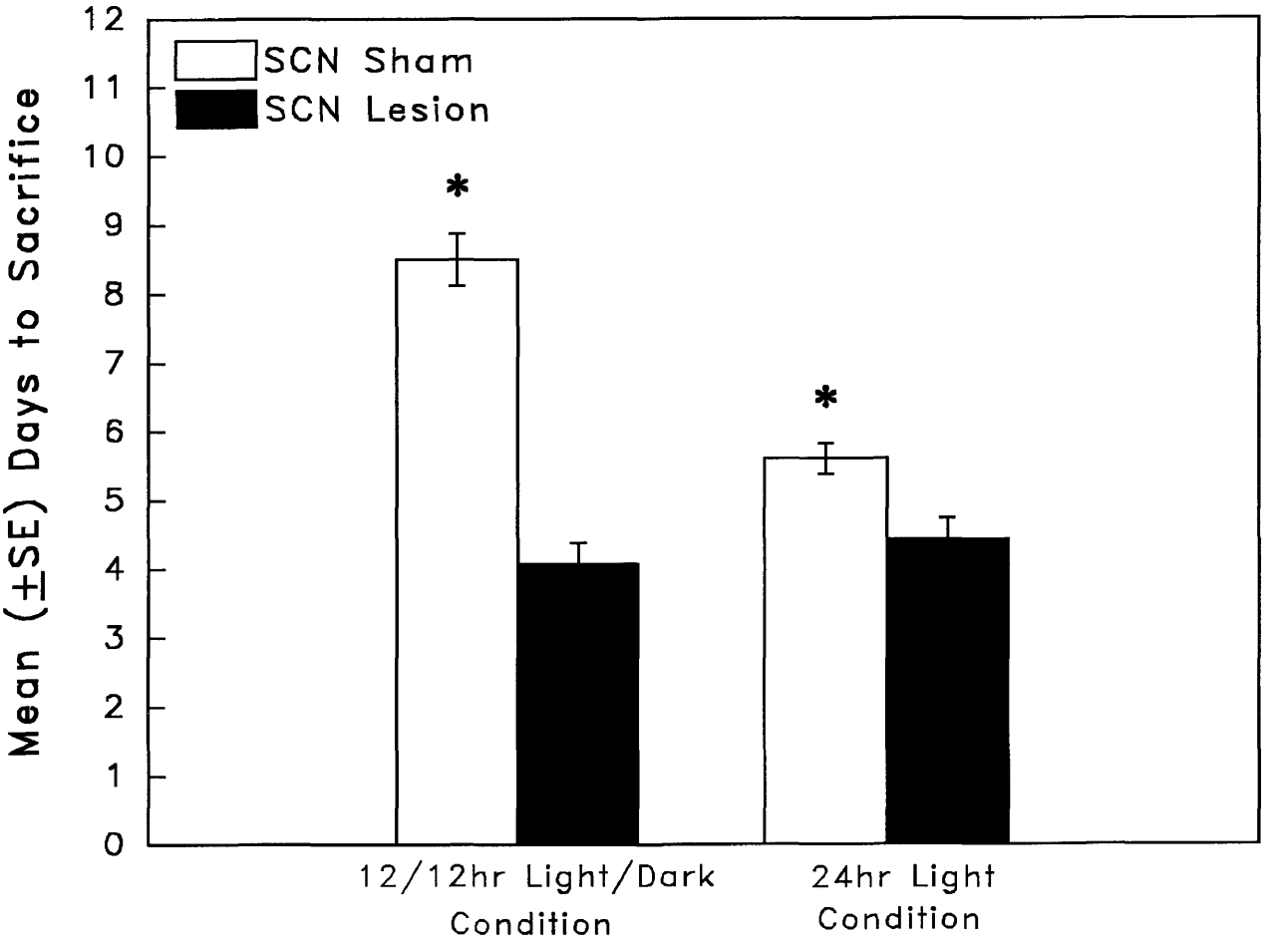
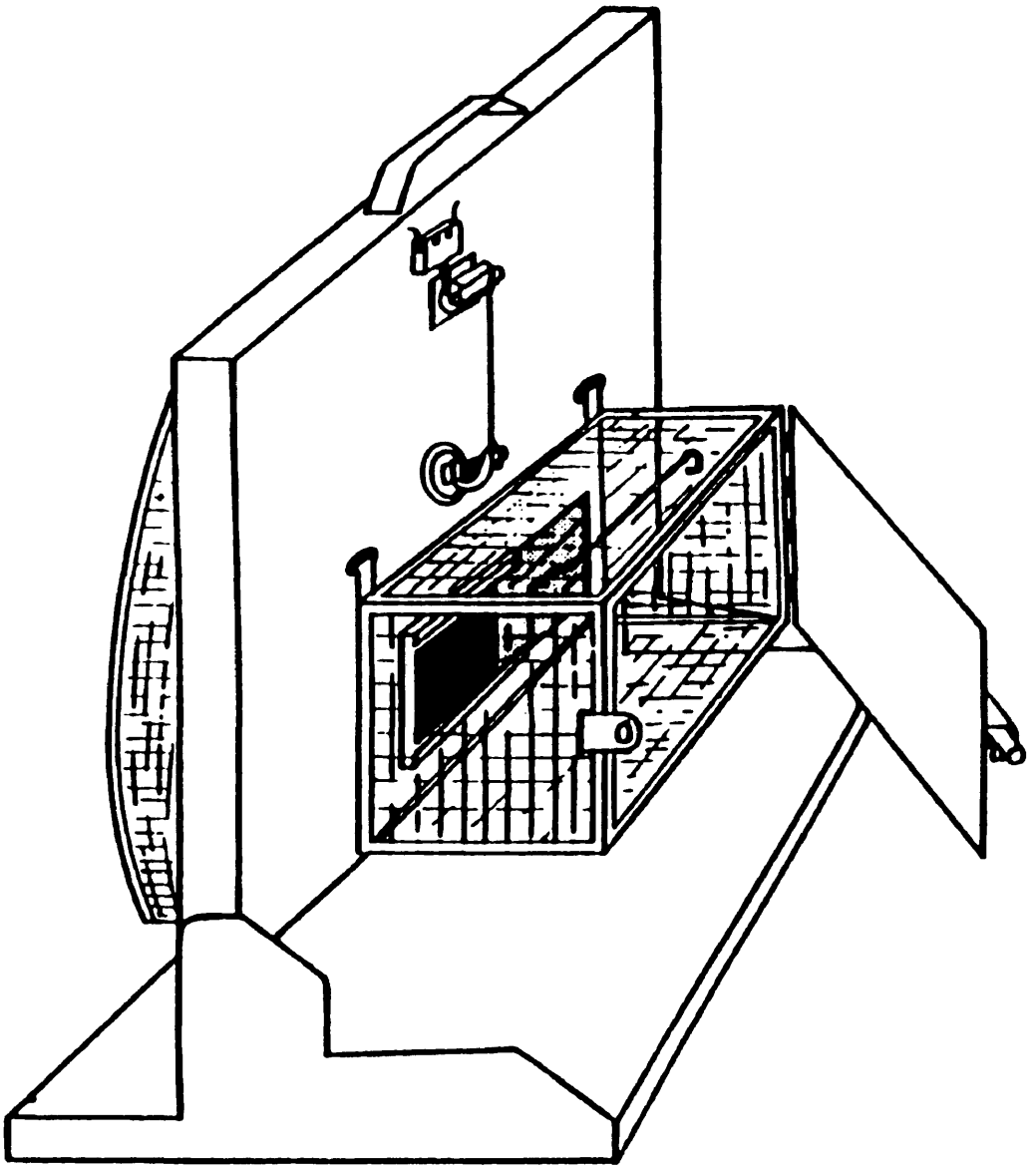

SCN Sham
SCN Lesion
Mean (±SE) Unilateral Adrenal Wt.
(g per kg of Body Wt.)
0.175
0.150
0.125
0.100
0.075
0.050
0.025
0.000
12/12hr Light/Dark
Condition
24hr Light
Condition

Figure Caption

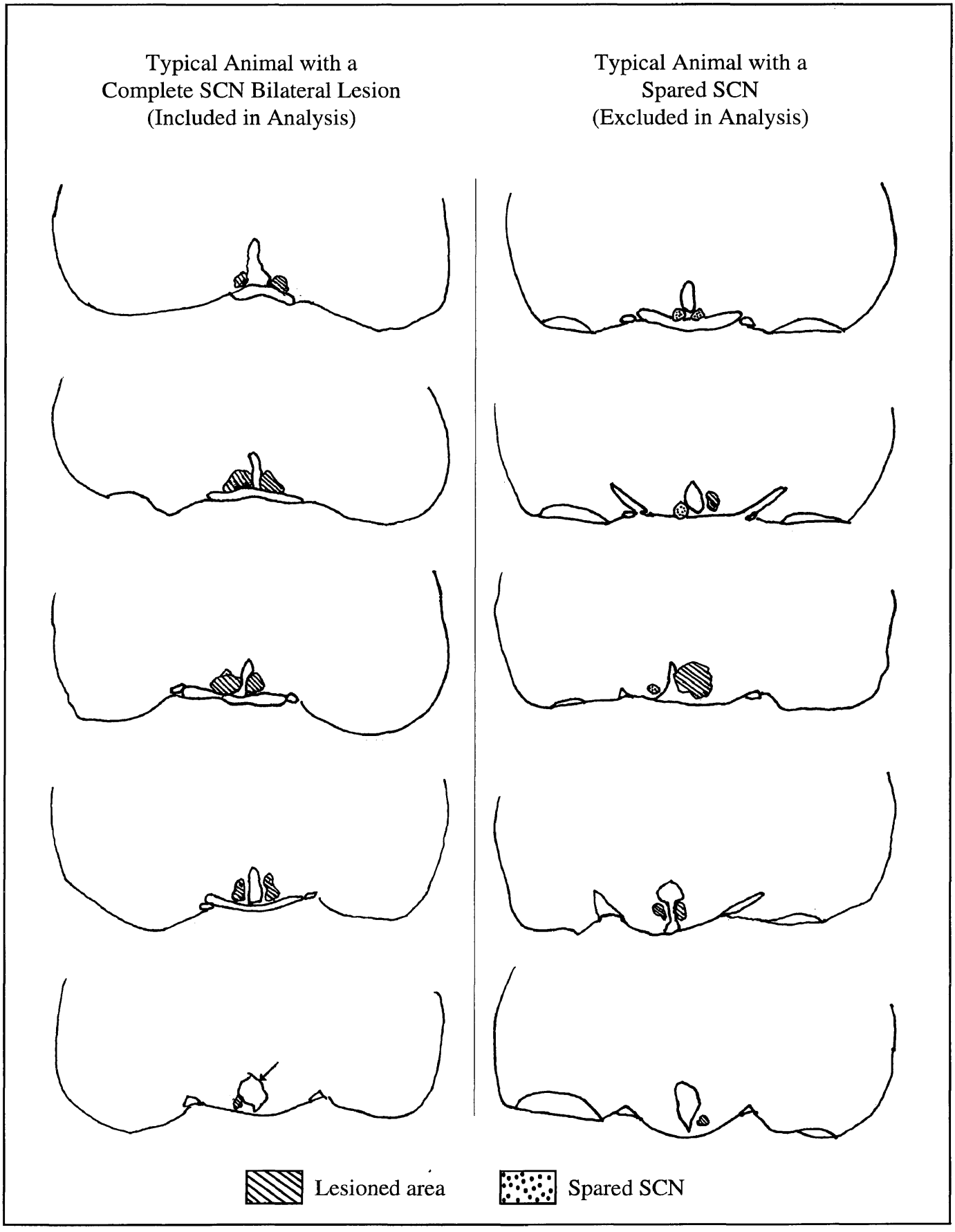
Figure 9. Mean $\pm$ SE number of days required to lose 25 percent of original body weight for the treatment conditions indicated in Figure 1. (* $p < .05$, differs significantly from all other groups)


SCN Sham
SCN Lesion
Mean (±SE) Days to Sacrifice
0
1
2
3
4
5
6
7
8
9
10
11
12
*
*
12/12hr Light/Dark Condition
24hr Light Condition

APPENDIX A



APPENDIX B


Typical Animal with a
Complete SCN Bilateral Lesion
(Included in Analysis)
Typical Animal with a
Spared SCN
(Excluded in Analysis)
Lesioned area
Spared SCN

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